

**GENERATION OF POLYUNSATURATED FATTY ACIDS FROM VEGETABLE
OILS USING THE LIPASE FROM GROUND OAT (*AVENA SATIVA* L.) SEEDS AS
A CATALYST**

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SUMMARY

Ground oat seeds can serve as a source of lipase in both aqueous-oil emulsions and in organic solvents. In organic solvents the lipase lost selectivity for monounsaturated fatty acids. Because of this, polyunsaturated vegetable oils were rapidly split. The lipase source is very inexpensive and is recyclable, to a certain extent.

INTRODUCTION

In industry, fats and oils are cleaved by a high-temperature steam treatment (Sontag, 1989). This process is energy intensive and also causes extensive degradation of polyunsaturated fatty acids (PUFAs). The PUFAs must be subjected to extensive purification procedures before they are suitable for use in production of important industrial chemicals such as dimer acids (Leonard, 1979), alkyd resins (Swern, 1964), and C₂₁ dicarboxylic acids (Ward *et al.*, 1975). Because of this degradation problem, in some industry sectors, polyunsaturated vegetable oils are cleaved using base followed by acidulation. This procedure does not achieve complete splitting.

Previously we showed that the buffer-extracted lipase from oat seeds is relatively heat stable and selectively cleaves oleic acid from tallow in a biphasic fat-water system (Piazza *et al.*, 1989). Here it is demonstrated that ground oat seeds can serve as a source of 'immobilized' lipase activity, and that when vegetable oil glycerides, containing in some cases very high levels of PUFAs, are treated with this lipase under appropriate reaction conditions, nearly complete splitting is obtained even at room temperature (RT).

Linfield *et al.*, 1984, reported the splitting of olive oil using *Candida rugosa* lipase, but successful hydrolysis required prior treatment of the oil with bleaching earth. Park *et al.*, 1988, reported the lipase-catalyzed lipolysis of soybean oil. Nearly complete lipolysis required the use of two lipases; each lipase, alone, could only catalyze partial splitting.

Recently, Lee and Hammond, 1990, reported the splitting of several vegetable oils with whole dehulled oat seeds. Lipolysis was conducted in oil without agitation. Achievement of over 90% lipolysis required 58 days, and three batches of seeds had to be added due to what was reported to be "inhibition by glycerol." The authors noted that better lipolysis could be achieved with the addition of solvent and with agitation of the oil, yet, surprisingly, no attempts were made to investigate the possibility of complete lipolysis using solvent and agitation.

MATERIALS AND METHODS

Oat seeds. Regal race horse oats were from U.S. Grain Co. (Timonium, MD).

Chemicals and materials. Soybean, corn, and olive oils were commercial products purchased from a local grocery store. Cotton seed oil, tricine, and gum arabic were from Sigma (St. Louis, MO). Diethylether was from Mallinckrodt (Paris, KY). Hexane was from Burdick and Jackson (Muskegon, MI). Aldrich Chemical (Milwaukee, WI) was the source of 2,2,4-trimethylpentane (TMP) and 1,1,2-trichlorotrifluoroethane. Bioact® 121, 123, and 255 were from Petroferm (Fernandina Beach, FL). Silica gel G thin layer plates were from Analtech (Newark, DE).

Lipase preparation. Oat seeds (4 g) were ground in a 37 ml Waring mini jar for 15 seconds. Endogenous oil lipids were extracted by stirring the ground oats twice with 75 ml diethylether for 30 minutes at RT. The diethylether was decanted, and the residual solvent was removed by placing the oats in a vacuum desiccator.

Oil lipolysis. All reactions were conducted in 125 ml Erlenmeyer flasks equipped with a glass stopper. Aqueous hydrolysis was typically conducted as follows. Vegetable oil (0.4 g), 10% gum arabic (8 ml), and 2M tricine (8 ml) were placed in the flask (final pH 9.0), and the mixture was sonicated to achieve a uniform emulsion. After the addition of defatted oats (4 g before defatting), the flask was shaken in a Controlled Environment Incubator Shaker (New Brunswick Scientific, New Brunswick, NJ) at 200 rpm. Lipolysis reactions in organic solvents typically contained defatted oats and vegetable oil as above in 16 ml organic solvent with 0.8 ml water. No sonication was required. The flasks were shaken as above.

Analysis of lipolysis products. As demonstrated in this manuscript the lipolysis products generated in organic solvent could be analyzed by thin-layer chromatography (TLC) without prior treatment. In aqueous media the following steps were taken. The pH of the reaction solution was first lowered to pH 3.0 by the addition of concentrated sulfuric acid. Then the lipids were extracted with organic solvent as described in the text. The TLC plates were developed and visualized, and gas-liquid chromatography (GLC) of the fatty acid fraction was conducted as previously described (Piazza *et al.*, 1989).

RESULTS

Grinding oat seeds. Very low lipolytic activity was observed with whole oat seeds. In a four hour RT experiment the use of ground oat seeds (8 g) increased observed fatty acid formation from undetectable levels to 0.24 g (approximately 2/3 of complete hydrolysis).

Defatting oat seeds. This was conducted to remove endogenous glycerides and fatty acids from the seeds in order to clarify TLC analysis. The ground oat seeds were defatted according to the procedure described in Materials and Methods using TMP, 1,1,2-trichlorotrifluoroethane, or diethylether. After removing all solvent from the ground seeds, lipolysis was conducted in TMP; no differences in the lipolysis rate were observed between the various treatments. In subsequent experiments diethylether was used as the defatting agent because it was easily removed from the oats.

Reaction conditions. Initial studies were conducted at RT. Lipolysis was observed when TMP, hexane, 1,1,2-trichlorotrifluoroethane and diethylether were used as solvents. Very low or no activity was detected in toluene, ethanol, isopropanol, dioxane, acetonitrile, Bioact® 121, 123, and 255. The amount of water added was found to be critically important. Little activity was noted with no added water, and activity began to decrease when 1.6 ml or more was added to 16 ml TMP containing 4 g defatted, ground oats (weight measured before defatting). Unlike the lipase activity in aqueous media, added calcium ion (CaCl_2) had no effect on the reaction rate in TMP. The pH optimum of extracted oat lipase lies between 7.5 and 9.0. If the ground oats were placed in water, the measured pH was 5.5. Tricine buffer was added to raise the pH to 8.0. The mixture was lyophilized and then utilized. No increase in the lipolysis rate was noted.

TLC was utilized in order to determine the minimum time for obtaining nearly complete lipolysis. As shown in Figure 1, directly spotting the solvent gave satisfactory results (lane C). No increase in observed fatty acid was noted when the sample was first acidified (lane D). When reactions were performed with soybean oil emulsified with gum arabic in water, the reaction rate was slow (lane E). Analysis of the fatty acids by GLC showed that the lipase had selectivity for monounsaturated fatty acids in aqueous media. In organic solvent very little selectivity for monounsaturated acids was noted (studies conducted with tallow). Since soybean oil contains over 50% PUFAs, the loss in lipase selectivity explains the faster lipolysis rate in TMP. As shown in lanes F, G, H, and I, cotton, olive, soybean, and corn oils are almost completely cleaved by the ground oat seed lipase in 48 hours at room temperature. The percents PUFAs in the fatty acid fractions of hydrolyzed cotton, olive, soybean, and corn oils were 48%, 5%, 57% and 34% of total, respectively.

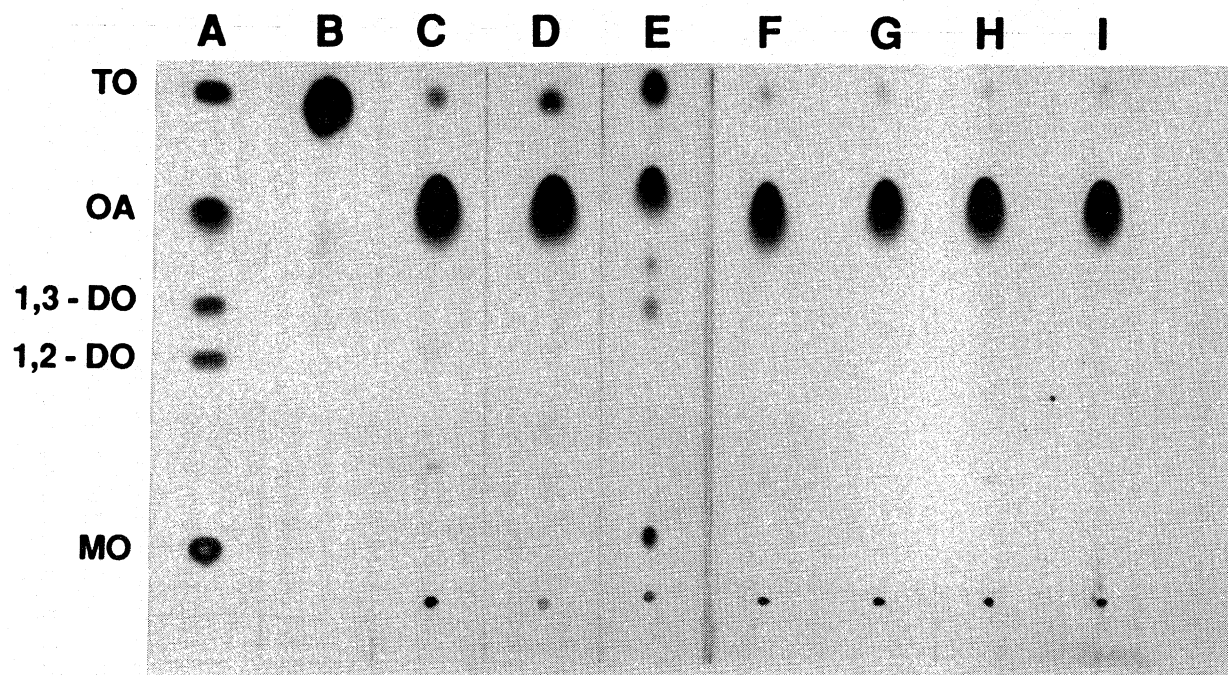


Figure 1. TLC analysis of the lipolysis of cotton, olive, soybean, and corn oils by the lipase of ground oat seeds at RT. Reactions contained 0.4 g vegetable oil, 16 ml TMP, 0.8 ml water, and 4 g defatted ground oat seeds (weight measured before defatting). Lane A. Standards: TO, triolein; OA, oleic acid; 1,3-DO, 1,3-diolein; 1,2-DO, 1,2-diolein; MO, monoolein. Lane B. Unreacted soybean oil. Lane C. Soybean oil plus lipase in TMP, 24 hour reaction time; sample taken with pipette and spotted directly. Lane D. Same as B, except 1.38 g sulfuric acid and 6.4 ml isopropanol were added to reaction. After shaking for 1 min the sample for TLC was taken. Lane E. Soybean oil plus lipase in an aqueous-gum arabic emulsion, 24 hour reaction time. Lanes F, G, H, I. Cotton, olive, soybean and corn oils plus lipase in TMP, 48 hour reaction time.

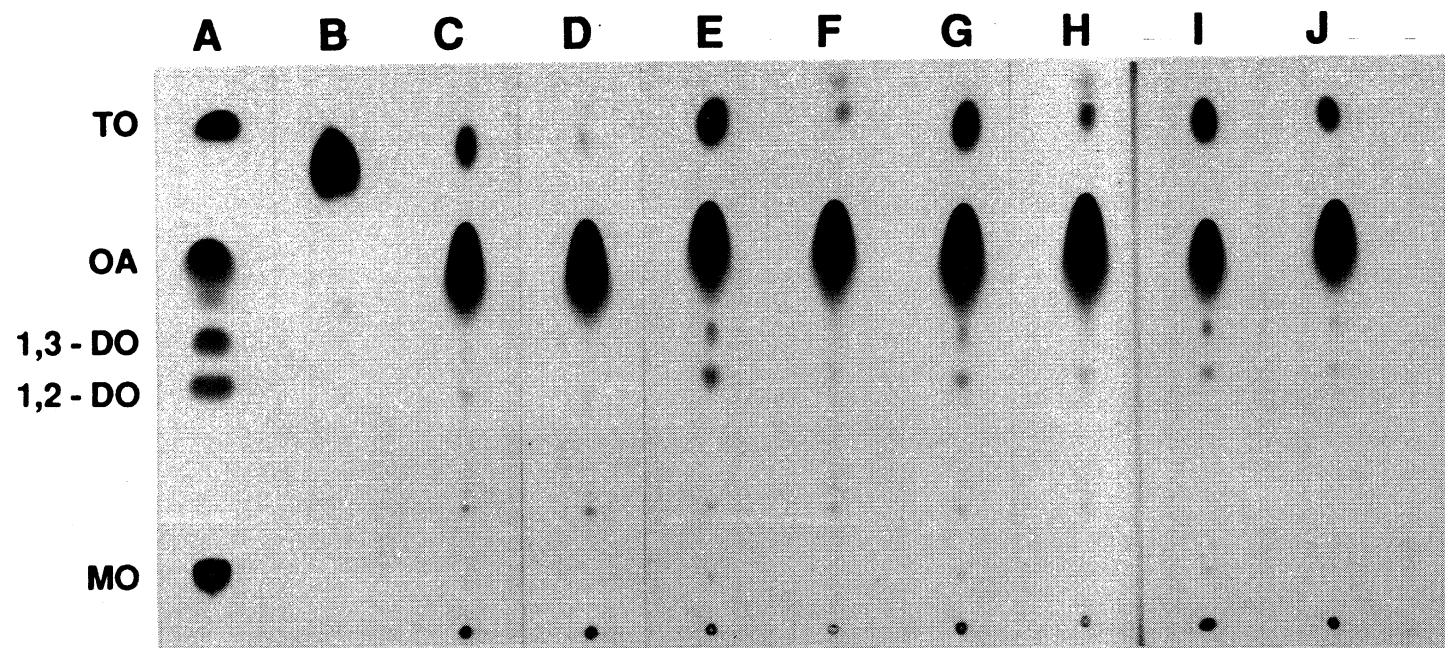


Figure 2. TLC analysis of the lipolysis of soybean oil by the lipase of ground oat seeds in TMP at various temperatures. Reaction conditions as in Fig. 1. Lane A. Standards, as in Fig. 1. Lane B. Unreacted soybean oil. Reaction temperatures and times: Lane C. 35°C, 6 hours. Lane D. 35°C, 19 hours. Lane E. 45°C, 6 hours. Lane F. 45°C, 19 hours. Lane G. 55°C, 6 hours. Lane H. 55°C, 19 hours. Lane I. 65°C, 6 hours. Lane J. 65°C, 19 hours.

The effect of temperature upon the rate of lipolysis was determined. As shown in Figure 2 (lanes B, C, and D) soybean oil is almost completely cleaved in only 19 hours at 35°C. Over the same time period elevating the temperature to 45°C, 55°C or 65°C gave poorer lipolysis (lanes E, F, G, H, I, and J).

The recyclability of the oat lipase was investigated. Lipolysis of soybean oil at 35°C in TMP was conducted. The TMP containing the PUFAs was decanted from the ground oat seeds. The oats were washed by shaking with 16 ml TMP for two minutes. Afterward a new solution of soybean oil in TMP was added to the oats, and lipolysis was followed by TLC. It was found that the lipolysis rate was roughly half of that observed during the first cycle. The addition of more water or CaCl_2 did not accelerate lipolysis, nor did washing with the solvents isopropanol, ethyl acetate, or diethyl ether. It was found that if the oats were first washed with TMP, dried, and then subsequently reground, the lipolysis rate during the second cycle was 90% of that observed in the first cycle.

CONCLUDING REMARKS

It is very important to achieve splitting at the lowest possible cost. Using the procedure described here, the cost of the oat seeds needed to break down a pound of oil is \$0.23 (retail). Since the oats can be reused, the actual operating cost might be lower. For industrial purposes it would not be necessary to defat the oats. This was carried out solely to clarify the TLC data. Although the use of an organic solvent in this procedure appears to rule it out as industrially viable, it is important to remember that seed oils are extracted with hexane. The rate of lipolysis in hexane is equal to that observed in TMP. TMP was utilized in these studies solely because it has a high boiling point, and this allowed experimentation at higher temperatures without extensive solvent loss due to evaporation.

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